Stabilizing Oils from Smoked Pink Salmon (*Oncorhynchus gorbuscha*)

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ABSTRACT: Smoking of meats and fish is one of the earliest preservation technologies developed by humans. In this study, the smoking process was evaluated as a method for reducing oxidation of pink salmon (*Oncorhynchus gorbuscha*) oils and also maintaining the quality of oil in aged fish prior to oil extraction. Salmon heads that were subjected to high temperatures (95 °C) during smoking unexpectedly produced oils with fewer products of oxidation than their unprocessed counterparts, as measured by peroxide value (PV), thiobarbituric acid reactive substances (TBARS), and fatty acids (FA). Higher temperatures and longer smoking times resulted in correspondingly lower quantities of oxidative products in the oils. Fatty acid methyl ester (FAME) analysis of smoke-processed oils confirmed that polyunsaturated fatty acids (PUFA) were not being destroyed. Smoke-processing also imparted antioxidant potential to the extracted oils. Even when antioxidants, such as ethoxyquin or butylated hydroxytoluene, were added to raw oils, the smoke-processed oils still maintained lower levels of oxidation after 14 d of storage. However, decreased antioxidant capacity of smoke-processed oils was noted when they were heated above 75 °C. Vitamin studies supported the antioxidant results, with smoke-processed oils displaying higher levels of α-tocopherol than raw oils. Results suggest that smoking salmon prior to oil extraction can protect valuable PUFA-rich oils from oxidation. Improved preservation methods for marine oils may extend their usefulness when added as a supplement to enhance levels of eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) in foods.

Keywords: Alaska salmon, fish oil, fishery by-products, lipid oxidation

Introduction

Alaska is responsible for over half the total fish landings in the United States, about 9% of which is salmon (Crapo and Bechtel 2003). Wild-caught Alaskan salmon possess high concentrations of long-chain n-3 polyunsaturated fatty acids (PUFA) that may not be present in farmed salmon raised on diets containing tropical oils such as palm (Bell and others 2002). Unsaturated lipids found in fish oils, have been associated with beneficial health effects (Hasler 2000), and consequently are appearing more often in foods. However, lipids with increased unsaturation are sometimes more susceptible to oxidative degradation (Nawar 1996). Vulnerability to lipid oxidation is known to vary among fish species as well as among different fish components. For example, crude oil extracted solely from herring heads had a lower stability than oil from mixed herring by-products (skins, frames, and viscera), which the authors attributed in part to the lower concentration in herring heads of α-tocopherols, naturally occurring antioxidants found in fish tissues (Aidos and others 2002).

Incorporating PUFAs into foods and feeds can decrease the oxidative stability of the final product (Augustin and Sanguansri 2003), which can result in loss of desirable flavors, colors, aromas, and nutritive properties, including the destruction of PUFAs and loss of fat-soluble vitamins. Oxidation of oils can also produce toxic compounds (Hultin 1994). Aqueous solutions were found to protect long-chain PUFAs, resulting in eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA), becoming more stable than shorter

chain fatty acids (FA) such as linoleic and linolenic (Miyashita and others 1993). This unexpected stability appears to be associated with the conformation of EPA and DHA in aqueous environments (Kato and others 1992). Oil-in-water emulsions were also found to provide oxidative stability for fish oils rich in long-chain PUFAs (Frankel and others 2002). A practical application of this concept was demonstrated in salad dressing, where emulsified oils were more effectively protected from oxidation than when straight fish oil was added (Let and others 2007).

Smoking fish has historically been used as a preservation method. In addition to imparting flavor and aroma attributes, smoke can protect foods from oxidation (Pearson and Gillett 1996; Schwanke and others 1996). Chemicals from wood smoke commonly include phenols, organic acids, alcohols, carbonyls, hydrocarbons, and nitrogen compounds such as nitrous oxide (Pearson and Gillett 1996). Among these components, phenols have demonstrated antioxidant capabilities (Pearson and Gillett 1996). Nitrites are also known to inhibit lipid peroxidation by participating in the radical-quenching process (Nicolescu and others 2004). The peroxidation process can also be inhibited by formate anion, which is a radical scavenger found in smoke flavorings and smoke-processed meats (Schwanke and others 1996; Coronado and others 2002).

As PUFA-enriched products become increasingly associated with enhanced nutritional value, new methods for controlling oxidative damage will emerge. When fish oil was added directly to pig feeds, higher levels of lipid oxidation occurred and "fishy" odors in the pork were detectable (Trout and others 1998). Bacon from pigs that received a dietary supplement of fishmeal experienced increased levels of oxidation (as measured by thiobarbituric acid reactive substances [TBARS]) during 4 mo of frozen storage. However, lipid oxidation was reduced when the pigs also received 200 mg α-tocopherol per kilogram of feed, and the bacon was processed with a combination of liquid and wood smoke (Coronado and others

2002). These findings suggest that smoke-processing fatty fish such as salmon may offer a solution to oxidation and off-flavors of PUFA-rich oils. The objective of this study was to examine the conditions of smoke-processing required to reduce oxidation of salmon oil during storage.

Materials and Methods

Salmon by-products

Pink salmon (*Oncorhynchus gorbuscha*) heads ($n = 108$, ranging from 113 to 608 g) were collected from a commercial processor located in the city of Kodiak, (Kodiak Island, Alaska, U.S.A.) in August 2007, and stored at -20 °C until shipped to Fairbanks (Alaska, U.S.A.) for processing. Samples were placed in a Bradley Smoker (Bradley Technologies Canada Inc., Richmond British Columbia, Canada), and smoked using hickory bisquettes. In one study, salmon heads were smoked at different temperatures (40, 60, 75, or 95 °C) for 5 h ($n = 9$ per treatment). In a 2nd study, salmon heads ($n = 9$ per treatment) were smoked at a constant temperature (95 °C) for different times (1, 2, 3, 4, or 5 h). Smoked salmon heads were processed using a Tor Rey F12-FS electric meat grinder (Tor Rey USA Inc., Houston, Tex., U.S.A.) with a 0.32-cm (1/8 inch) plate. Homogenized salmon heads were placed into 50-mL Corning centrifuge tubes for oil extraction (16500 × g; 20 min; 4 °C) using a Beckman J2-HS centrifuge (Fullerton, Calif., U.S.A.) equipped with a JA-20 rotor. Oil was removed from each tube, pooled, and placed into amber glass vials, flushed with nitrogen and stored frozen (-80 °C). Cooked oils were obtained by homogenizing 10 raw salmon heads and heating the ground tissue in a 95 °C water bath for 55 min. Oil was extracted and stored as described previously.

Shelf life of smoked salmon heads and extracted oils

Oils (1 g) extracted from smoked and raw salmon heads were stored in small (1.5 mL) loosely capped vials at 2 temperatures (4 or 35 °C) for up to 30 d to assess storage stability. Three vials were removed daily from each treatment group and tested for indicators of oil quality (FA, PV, TBARS). Intact salmon heads were also stored up to 6 d at 2 different temperatures (4 or 35 °C) prior to oil extraction to compare storage stability of oils within raw and smoke-processed salmon heads. Alpha-tocopherol (vitamin E) levels were measured in both raw and smoked salmon oils stored for 0 and 30 d (4 and 35 °C), as well as in oils extracted on day 6 from intact salmon heads stored at 4 and 35 °C. Vitamin E values were also determined for salmon heads smoke-processed (5 h) at different temperatures (40, 60, 75, or 95 °C).

Compositional analysis

Three samples from each treatment were analyzed in duplicate for each of the following analyses. Moisture was determined gravimetrically by drying samples for 24 h and measuring water loss (method 952.08, AOAC 1990). Protein was measured by drying samples and analyzing for nitrogen content on an Elementar Rapid NIII analyzer (Mt. Laurel, N.J., U.S.A.), which multiplies nitrogen values by a conversion factor of 6.25 (based on the protein to nitrogen ratio of bovine serum albumin) to calculate protein values. Lipids were determined by processing dried samples on a Soxtec Model 2043 (method 991.36, AOAC 1990) using a methylene chloride extraction solvent (12 mL/g), after which, lipid-rich solutions were evaporated to dryness to remove solvent, and then weighed. Ash content was determined by placing samples into a muffle furnace at 550 °C for 24 h and then weighing the remaining material (method 938.08, AOAC 1990).

Fatty acids

FA were analyzed in triplicate according to Bernárdez and others (2005). In brief, approximately 50 mg of oil were dissolved in 1.5 mL of cyclohexane and 0.5 mL of 5% (w/v) cupric acetate (adjusted to pH 6 with pyridine) was then added and vortexed for 30 s. Samples were centrifuged for 10 min at 2000 × g and the absorbance of the upper layer (cylcohexane) read at 710 nm. Results were measured in milligrams per gram oil using oleic acid as the standard.

Peroxide values

Lipid peroxide values (PV) were determined in triplicate using the Intl. Dairy Federation iron-based method (IDF 1991), where oil (10 mg) was first dissolved in 9.8 mL of a 7:3 chloroform–methanol mixture, then vortexed prior to the addition of 30% ammonium thiocyanate solution (50 μL). After vortexing for 2 to 4 s, 50 μL of iron(II) chloride solution were added and vortexed. Samples were incubated at room temperature for no more than 5 min and absorbances were immediately read at 500 nm. Results were expressed in milliequivalents peroxide per kilogram oil using the equation: $PV = (\mu g\ Fe^{3+})/(55.84)(m)/(2)$, where m is the mass of oil in grams, and the ferrous ions (Fe^{3+}) in the crude oil were calculated from a standard curve.

Thiobarbituric acid reactive substances

TBARS were determined in triplicate according to Siu and Draper (1978) with slight modifications by dissolving 50 mg of oil in 3.5 mL cyclohexane and 4.5 mL of 7.5% trichloroacetic acid (TCA) containing 0.34% thiobarbituric acid (TBA). The TCA minimizes interfering solubles by acid-precipitating the lipoprotein fractions. Samples were mixed for 5 min (to allow the secondary lipid oxidation products from the oil to dissolve into the polar layer), and then centrifuged for 15 min at 1555 × g. The aqueous TCA–TBA phase was separated from the nonpolar solvent, and incubated at 100 °C for 10 min while the Malonaldehyde (MDA) and TBA reacted to form a chromogen, which was detected at 532 nm using a SpectroMax Plus microplate spectrophotometer (Molecular Devices, Union City, Calif., U.S.A.). The intensity of color correlated with the quantity of TBARS (principally MDA) in the oxidized oil, and was reported as milligrams per kilogram oil using malonaldehyde-bis as the standard. Although the smoked-oil samples in this study acquired progressively darker colors with increased smoking times and temperatures, full-spectrum absorbance scans revealed no interfering compounds at 532 nm.

Fatty acid methyl esters preparation and analysis

Crude salmon oils from each treatment were stored under nitrogen at -80 °C until derivatization. Methyl esters were prepared according to the procedure of Maxwell and Marmer (1983) using 23:0 as internal standard. Fatty acid methyl esters were separated and quantified as described by Bechtel and Oliveira (2006). Briefly, an Agilent Technologies model 6850 gas chromatograph (GC) (Wilmington, Del., U.S.A.) equipped with a flame ionization detector (FID) and a DB-23 (60 m × 0.25 mm id., 0.25 μm film) capillary column (Agilent Technologies) was used for separation and quantification of fatty acid methyl esters. Hydrogen was used as carrier gas at a constant flow of 1 mL/min. Detector and injector were held at a constant temperature of 275 °C, and the split ratio was 25:1. The oven programming was 140 to 200 °C at a rate of 2 °C/min, 200 to 220 °C at a rate of 0.5 °C/min, and 220 to 240 °C at a rate of 10 °C/min for a total run time of about 62 min. An autosampler performed the injections of standards and samples at a constant volume of 1 μL. Data were collected and analyzed using the GC ChemStation program (Rev.A.08.03 [847]; Agilent Technologies,

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1990–2000). All standards used in the identification of peaks were purchased from Supelco® (Bellefonte, Pa., U.S.A.). The standards used were: Supelco® 189-19, bacterial acid methyl esters mix, marine oil nr 1, and marine oil nr 3.

Measuring antioxidant potential

A photochemiluminescence (PCL) detection method using a Photochem (Analytik-Jena, Jena, Germany) was used to quantify lipid soluble antioxidants. The antioxidant potentials were measured using the antioxidative capacity of lipid-soluble substances (ACL) kit and procedures provided by the manufacturer (Analytik Jena AG 2005). Triplicate samples of approximately 50 mg of extracted oil were solubilized in 1 mL of n-hexane (Fisher Scientific, Pittsburgh, Pa., U.S.A.) and filtered through a 0.45 μm disk filter. A portion of the solubilized oil was used to measure antioxidant potential 3 times. Units were reported as Trolox Eq/g oil. Quantification was based on a calibration curve developed from Trolox.

Evaluating resistance to oxidation

Triplicate samples of raw and cooked salmon oils were supplemented with commercially available antioxidants, butylated hydroxytoluene (BHT) or ethoxyquin (EQ) to serve as controls when evaluating the effectiveness of smoked oils in resisting oxidation. BHT and EQ solutions were prepared by adding 0.01% into 100 mL MeOH. Oils (1 g) were incubated at 21 °C for 0, 1, 7, and 14 d. Each day, triplicate samples were placed in a vacuum oven (12 h, 35 °C) to remove methanol, then flushed with nitrogen and stored at −80 °C until oxidation analysis (TBARS, FAs, and PVs).

Tocopherols

The compound α-tocopherol (vitamin E) was measured in triplicate according to the method of Wu and Bechtel (unpublished data) using high-performance liquid chromatography–atmospheric pressure chemical ionization–mass spectrometry (HPLC–ACPI–MS) (Agilent, Palo Alto, Calif., U.S.A.) in selective ion mode (SIM) equipped with a zorbax C18 column. A gradient elution was used on the HPLC. The mobile phase was methanol:water (97:3) held for 15 min and ramped up to methanol (100%) at 20 min and held for 4 min. Oil samples were dissolved in 2-propanol (1 mL). The conditions for the mass spectrometry detector were as follows: 200 V for the fragmentor, 5 L/min for the drying gas, 60 psig for the nebulizer pressure, 350 and 250 °C for the drying gas and vaporizer temperatures, 3000 V for the capillary voltage, and 4.0 μA for the corona current. Quantification was based on a calibration curve with α-tocopherol detected with ion 431.3.

Statistical analysis

The effect of treatment was investigated using one-way analysis of variance (ANOVA) conducted with the Statistica version 7.1 software package (Statsoft, Tulsa, Okla., U.S.A.). The ANOVA *P*-value was set to 0.05, and differences between treatments were examined using the post hoc test Tukey's equal *N* honestly significant differences ($P < 0.05$). FA concentrations included in the ANOVA analysis had a minimum abundance of 20 mg.

Results and Discussion

Composition of smoked salmon heads

The composition of unprocessed and smoked salmon heads are listed in Table 1. Carbohydrates are not included since they are negligible in fish tissues (Oliveira and Bechtel 2005). The heat treat-

ment incurred by the samples during smoke-processing (95 °C, 5 h) resulted in predictably lower moisture content (53.8%) than found in unprocessed salmon heads (70.1%). Consequently, the percentages of protein, lipid, and ash appear to be higher in the smoked samples, even though all samples were similar on a dry matter basis (52% lipid, 41% protein, and 7% ash).

Effects of time and temperature on oxidation

Oil extracted from pink salmon heads that were subjected to high temperatures (during smoking or during regular 95 °C open-vat heating) unexpectedly produced fewer products of lipid oxidation than their unprocessed counterparts, as measured by PV, TBARS,

Table 1 — Compositional analysis of untreated and smoke-processed pink salmon heads (means and standard errors).

	Moisture (%)	Protein (%)	Lipid (%)	Ash (%)
Raw salmon	70.1 ± 0.4	12.3 ± 0.4	14.8 ± 0.4	2.3 ± 0.1
Smoked salmon	53.8 ± 1.4[a]	17.4 ± 0.2[a]	24.3 ± 0.9[a]	2.8 ± 0.2[a]

[a]Significantly different ($P < 0.05$).
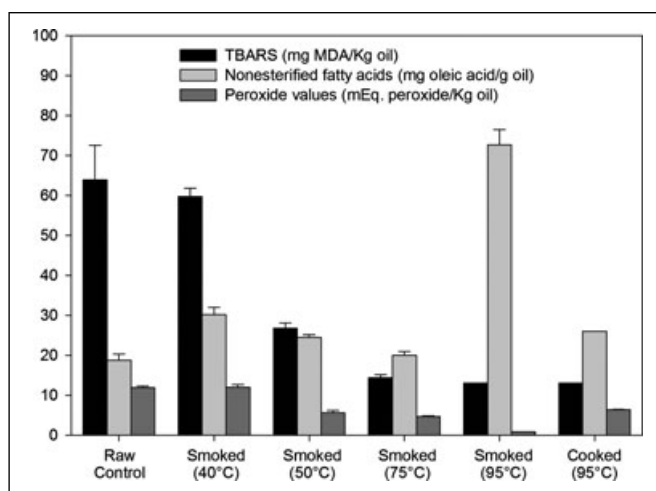
Figure 1 — Effect of different temperatures (40 °C to 95 °C) applied to salmon heads during smoke-processing (5 h) on the quality indicators (TBARS, fatty acids, PV) of extracted salmon oils.
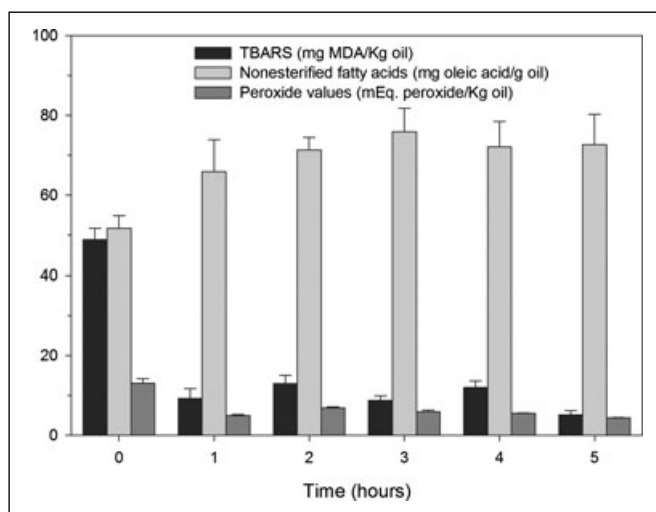

Figure 2 — Effect of different heating times (1 to 5 h) applied to salmon heads during smoke-processing (95 °C) on the quality indicators (TBARS, fatty acids, PV) of extracted salmon oils.

and FA values. Higher temperatures and/or longer smoking times resulted in correspondingly lower quantities of oxidative products (except FA) in the oils.

Evaluating lipid oxidation is complex since the oxidative breakdown products are unstable during extraction, storage, and analysis. To compensate, 3 different analytical methods based on 3 different principles were used in this study. Figure 1 demonstrates the relationship between the temperature of smoke-processing and

the resultant oxidation of the oils extracted from smoked salmon heads. Unsaturated FA involved in free-radical mediated oxidative processes are damaged when hydrogen is abstracted from a weak bis-allylic position on the fatty acid chain, and once oxidation has been initiated, radicals form and are rapidly combined with molecular oxygen to produce peroxyl radicals (Nawar 1996). The PV assay is used to measure primary oxidation by detecting changes in hydroperoxide concentrations. Salmon heads smoked
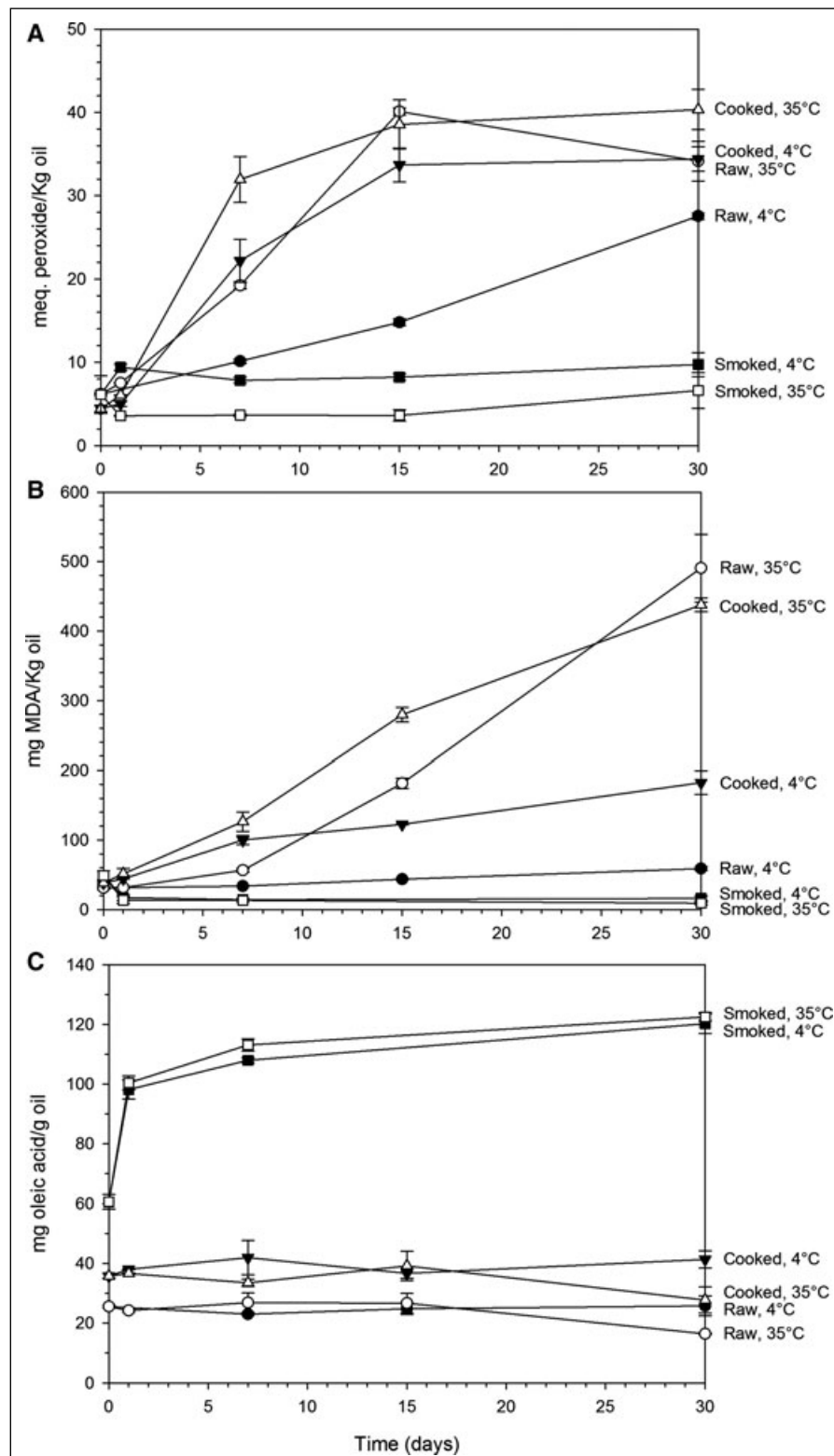

Figure 3 — Effect of smoke-processing on 30-day shelf life of oils extracted from smoked salmon heads after storage at 4 °C or 35 °C, as measured by (A) peroxide values, (B) TBARS, and (C) fatty acids.

at a low temperature (40 °C) produced oils with PV quantities similar to raw oils. As smoking temperatures increased, the extracted oils demonstrated progressively lower PV results, decreasing from 12 mEq peroxide/kg oil in the raw control to 2 mEq/kg in the oils extracted from 95 °C smoked salmon heads. When salmon heads were smoked at 95 °C for different times (1 to 5 h), PV did not decrease in a linear manner. However, PV results were significantly lower in all smoked samples when compared to the raw samples (Figure 2).

Hydroperoxides are transitory and rapidly break down into secondary products of oxidation, such as epoxides, hydroxyls, aldehydes, ketones, alcohols, and hydrocarbons. The TBARS assay is used to monitor oxidation by screening for aldehydes and other relatively polar or charged reaction intermediates (Nawar 1996). Malonaldehyde is one of the TBA reactive substances formed when PUFAs are oxidized (Hultin 1994) and is used to screen for oxidation of fish oils since normal tissues contain low MDA levels. In this

Figure 4 — Effect of smoke-processing on oils extracted from intact smoked salmon heads stored at 4 °C or 35 °C for up to 8 days as measured by (A) peroxide values, (B) TBARS, and (C) fatty acids.
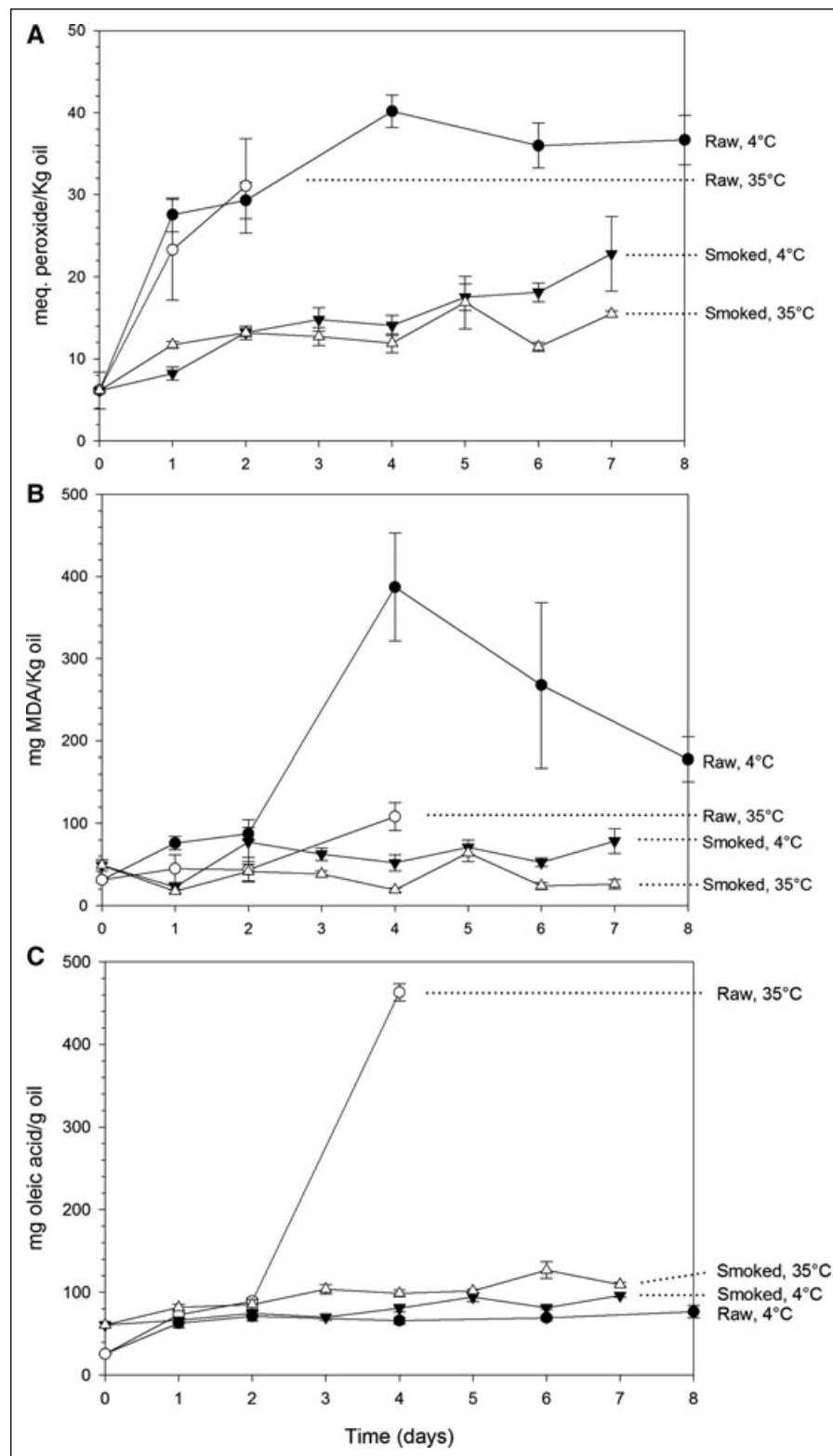
study, the levels of TBARS decreased with increasing temperatures (Figure 1), suggesting that the heating and/or smoking processes may inhibit oxidation of extracted oils. TBARS values also were lowest after the salmon heads had been subjected to the longest (5 h) smoking time (Figure 2).

Another method for detecting lipid oxidation involves the measurement of FA, which are formed as secondary products during lipid oxidation, as well as through hydrolytic cleavage when FA are freed from the parent triacylglycerol structure (Nawar 1996). In this study, heat-processed salmon heads (cooked or smoked) generally produced oils containing more FA than oil extracted from the raw salmon controls (Figure 1). High temperature smoke-processing (95 °C) resulted in the highest fatty acid values. However, increased smoking times at 95 °C did not exert an appreciable change in fatty acid levels among the samples (Figure 2). Crude fish oils, in their natural state, contain some FA, which may have contributed to the variation among samples.

Shelf life of oils extracted from smoked salmon

Shelf life stability studies were conducted on extracted salmon oils that were stored for up to 30 d before testing. PV for raw oils increased steadily from 7 mEq/kg to approximately 28 mEq/kg when stored under refrigeration (4 °C) for 30 d (Figure 3A). Raw oils stored at 35 °C accumulated higher levels of oxidation products (approximately 40 mEq/kg) within 15 d before decreasing to 30 mEq/kg by day 30. Oils extracted from cooked salmon heads and stored for 30 d also had high PV, stabilizing near 32 mEq/kg for refrigerated samples and 40 mEq/kg for those held at 35 °C during storage. In contrast, oils extracted from smoked salmon and then stored for 30 d maintained PV less than 10 mEq/kg for both storage temperatures, suggesting that less lipid oxidation was occurring compared to their raw and cooked counterparts.

TBARS assays also demonstrated increasing products of oxidation over 30 d of storage for oils extracted from raw and cooked salmon heads, compared to oils extracted from smoke-processed salmon heads (Figure 3B). The storage temperature had a significant effect on the quantity of oxidation products detected, with a higher storage temperature (35 °C) being associated with more products of lipid oxidation than lower temperatures (4 °C) in all samples tested. Oils extracted from raw and cooked salmon heads, when stored for 30 d at 35 °C had over 400 mg MDA/kg oil. Refrigerated storage resulted in lower TBARS values (160 mg MDA/kg oil for cooked salmon head oils and 50 mg MDA/kg oil for raw oils). However, the oils from smoked salmon heads had consistently lower TBARS (< 20 mg MDA/kg) over 30 d of storage, regardless of temperature. Lipid oxidation as measured by PV and TBAR values were in general agreement.

Fatty acid analyses showed an opposite trend with higher values occurring in oils extracted from smoked salmon (120 mg oleic acid per gram oil), as compared to oils from cooked (less than 40 mg/g oil) and from raw (less than 30 mg/g oil) salmon heads during 30 d of storage (Figure 3C). Variations in fatty acid quantities were similar among treatment groups regardless of temperature.

Stability of oils stored within intact smoked salmon heads

Shelf life stability studies were also conducted on whole salmon heads stored for up to 8 d before the oil was removed for testing. Raw salmon heads experienced rapid decomposition during storage, which resulted in progressively lower oil yields. No oil was recoverable by day 4 from samples stored at 35 °C, and none from 4 °C samples by day 6. This problem was not seen with smoke-processed samples.

PV of oils extracted after refrigerated storage of raw salmon heads increased to 38 mEq/kg by day 4 and remained at that level until day 8, when they were discarded (Figure 4A). Oils obtained from smoked salmon heads had substantially lower PVs, never exceeding 20 mEq/kg throughout storage. Refrigerated salmon,

Table 2 – Summary of fatty acid analysis of pink salmon oils (% w/w).

	Salmon oils (day 0)		Salmon oils (day 30, 4 °C)		Salmon oils (day 30, 35 °C)		Salmon heads (day 6)		
	Raw	Smoked	Raw	Smoked	Raw	Smoked	Raw (4 °C)	Smoked (4 °C storage)	Smoked (35 °C storage)
$\sum$ SAT (S)	21.4 ± 1.0^a	18.7 ± 0.3^a	23.3 ± 1.2^a	19.4 ± 0.9^a	20.6 ± 0.2^a	20.5 ± 0.4^a	20.4 ± 0.5^a	23.1 ± 0.9^a	22.7 ± 1.5^a
$\sum$ MUFA	40.2 ± 1.4^a	39.5 ± 1.0^a	45.5 ± 1.6^a	42.6 ± 3.3^a	38.8 ± 1.9^a	41.3 ± 0.7^a	41.5 ± 0.5^a	37.5 ± 1.3^a	38.7 ± 2.5^a
$\sum$ PUFA (P)	30.8 ± 0.1^{ab}	31.3 ± 1.9^{ab}	34.3 ± 0.4^a	32.9 ± 0.3^{ab}	24.2 ± 1.2^c	32.5 ± 0.2^{ab}	28.1 ± 1.3^{bc}	32.9 ± 0.6^{ab}	32.5 ± 1.4^{ab}
P/S	1.4	1.8	1.0	1.4	1.5	1.6	1.6	1.4	1.4
$\sum$ ω-3	28.5 ± 0.2^{ab}	28.2 ± 1.9^{ab}	32.2 ± 0.1^a	29.9 ± 0.3^{ab}	21.7 ± 1.2^c	29.5 ± 0.2^{ab}	25.5 ± 1.4^{bc}	29.5 ± 0.6^{ab}	29.9 ± 1.1^{ab}
$\sum$ ω-6	2.3 ± 0.1^{ab}	3.1 ± 0.1^{ab}	2.1 ± 0.3^b	3.0 ± 0.1^{ab}	2.4 ± 0.1^{ab}	3.0 ± 0.1^{ab}	2.6 ± 0.2^{ab}	3.4 ± 0.3^a	2.6 ± 0.4^{ab}
$\sum$ ω-3/ $\sum$ ω-6	12.4	9.1	15.6	9.9	8.9	9.9	9.6	8.7	11.6

SAT = saturated fatty acids; MUFA = monounsaturated fatty acids; PUFA = polyunsaturated fatty acids; P/S = ratio polyunsaturated to saturated fatty acids.
[abc]Different letters within a row indicate statistical differences at $P < 0.05$.

Table 3 – Effect of smoking temperature on oils extracted from smoke-processed and cooked salmon heads as evaluated by fatty acid methyl ester GC analysis as percent of total oil extracted.

	Smoked (40 °C, 5 h)	Smoked (50 °C, 5 h)	Smoked (75 °C, 5 h)	Smoked (95 °C, 5 h)	Cooked (95 °C, 1 h)
SAT %	20.3 ± 1.6^a	21.9 ± 1.2^a	20.4 ± 0.1^a	18.7 ± 0.3^a	20.1 ± 1.9^a
MUFA %	39.7 ± 2.5^a	38.0 ± 1.3^a	41.0 ± 0.2^a	39.5 ± 1.0^a	41.3 ± 1.9^a
PUFA %	31.9 ± 1.0^{ab}	34.2 ± 1.9^a	34.3 ± 1.2^a	31.3 ± 1.9^{ab}	32.4 ± 0.2^{ab}
P/S	1.7	1.6	1.6	1.8	1.6
ω-3%	29.3 ± 0.7^{ab}	32.0 ± 1.9^a	31.6 ± 0.8^a	28.2 ± 1.9^{ab}	30.1 ± 0.2^{ab}
ω-6%	2.6 ± 0.3^{ab}	2.2 ± 0.1^b	2.7 ± 0.4^{ab}	3.1 ± 0.1^{ab}	2.3 ± 0.1^{ab}
ω-3/ω-6	11.3	14.7	11.8	9.1	13.0

SAT = saturated fatty acids; MUFA = monounsaturated fatty acids; PUFA = polyunsaturated fatty acids; P/S = ratio polyunsaturated to saturated fatty acids.
[abc]Different letters within a row indicate statistical differences at $P < 0.05$.

whether raw or smoked, produced oils with slightly higher PV levels than salmon stored at 35 °C.

Oxidation of oils within stored salmon heads (as measured by TBARS) had trends similar to the PV results. Raw salmon heads stored at 35 °C produced oils with values of 100 mg MDA/kg oil on day 4 before tissue decomposition limited oil extraction (Figure 4B). Oils extracted from raw salmon heads stored at 4 °C reached TBARS values of 400 mg MDA/kg by day 4 before decreasing to 200 mg MDA/kg at day 8. Products of oxidation were lower in oils from smoke-processed salmon, less than 90 mg MDA/kg, with higher temperature storage (35 °C) consistently showing lower TBARS values. However, it is possible that oxidation products such as malondialdehyde may have bound to proteins in the surrounding tissues before oil extraction resulting in low TBARS values (Hultin 1994).

Raw salmon heads stored at 35 °C produced low quality oils as measured by high fatty acid levels (Figure 4C). Smoked salmon heads also suffered some deterioration during storage, although the fatty acid levels in the extracted oils were lower than their raw counterparts for both storage temperatures. In general, high-temperature smoke-processing of salmon heads appears to contribute to more stable oils with increased capability for storage. Similar temperature results were reported in a study in which oil extracted with heat (95 °C) from Atlantic salmon by-products was found to be stable for 120 d at refrigerated temperatures (4 °C), but not at higher storage temperatures (23 °C), (Skåra and others 2004).

Stability of PUFA during smoking and storage

Fatty acid methyl esters (FAMEs) of oils extracted from smoke-processed salmon heads were evaluated to determine whether components in smoke actually protected the oils from oxidation or merely destroyed (or bound) the measurable oxidized lipid fractions, resulting in artificially low TBARS and PV levels. As expected, oils extracted from raw salmon heads and then stored at 35 °C experienced significant loss of PUFAs by day 30 as compared to day 0 oils (Table 2). However, PUFA quantities in oils extracted from smoked salmon were not significantly different from day 0 raw oils, suggesting that unsaturated FA were spared by smoke-processing. Oils extracted 6 d after salmon heads were smoked retained PUFA levels comparable to fresh raw oils, regardless of storage temperature. Different smoke-processing temperatures (40 to 95 °C) also

had no effect on fatty acid levels nor did the oils differ significantly from fresh raw salmon oils (Table 3).

EPA is a 20-carbon fatty acid with 5 double bonds (C20:5n-3). Eicosanoids are derived from dietary FA and can act as hormone-like compounds (Lands 2005). The balance of FA in the linolenate (n-3) and linoleate (n-6) groups has been associated with inflammatory processes leading to disease (Lands 2005). Although the optimum dietary ratio of n-3 to n-6 FA remains controversial, it has been suggested that diets higher in n-3 PUFAs may offer a lower mortality risk from coronary heart disease (Leaf 2008). In this study, smoke-processing did not compromise the favorable n-3 to n-6 balance (Table 2 and 3).

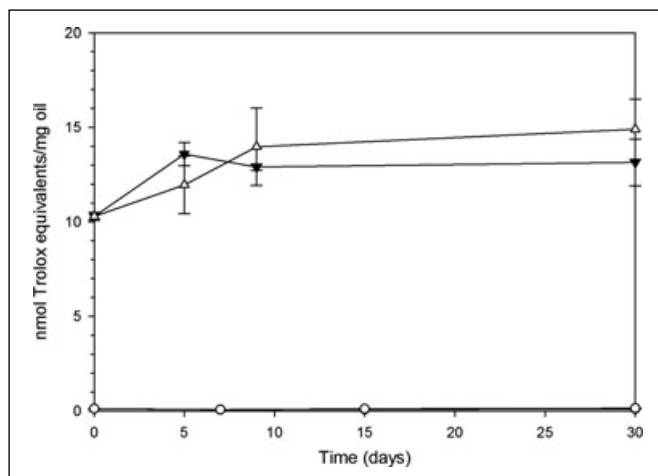## Antioxidant potential of oils from smoked salmon

Smoke-processing of salmon heads appears to impart antioxidant capabilities to the oils, since raw salmon oils displayed very low levels of antioxidant potential (Figure 5). A similarly low antioxidant potential was reported by Wu and Bechtel (unpublished data) for raw pink salmon oil (0.9 nmol Trolox Eq/mg of oil). Smoke-
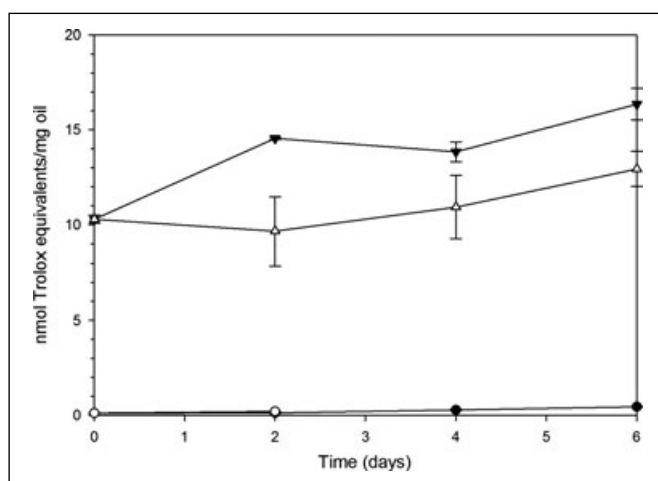
Figure 6 — Antioxidant potential of oils, stored for up to 6 days before extraction from raw salmon heads stored at 4 °C (●), raw salmon heads stored at 35 °C (○), smoked salmon heads stored at 4 °C (▼), and smoked salmon heads stored at 35 °C (△).
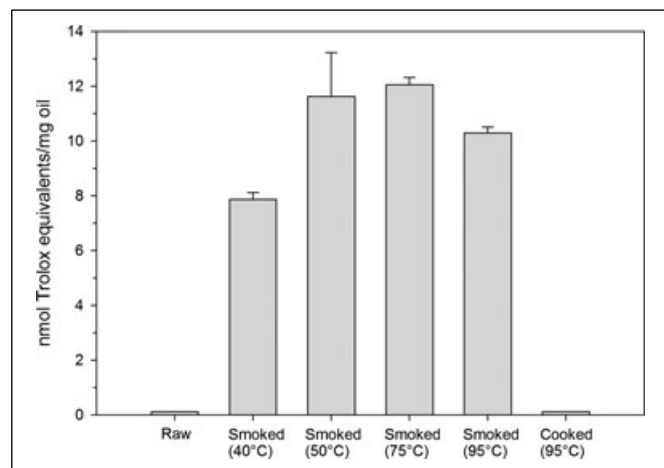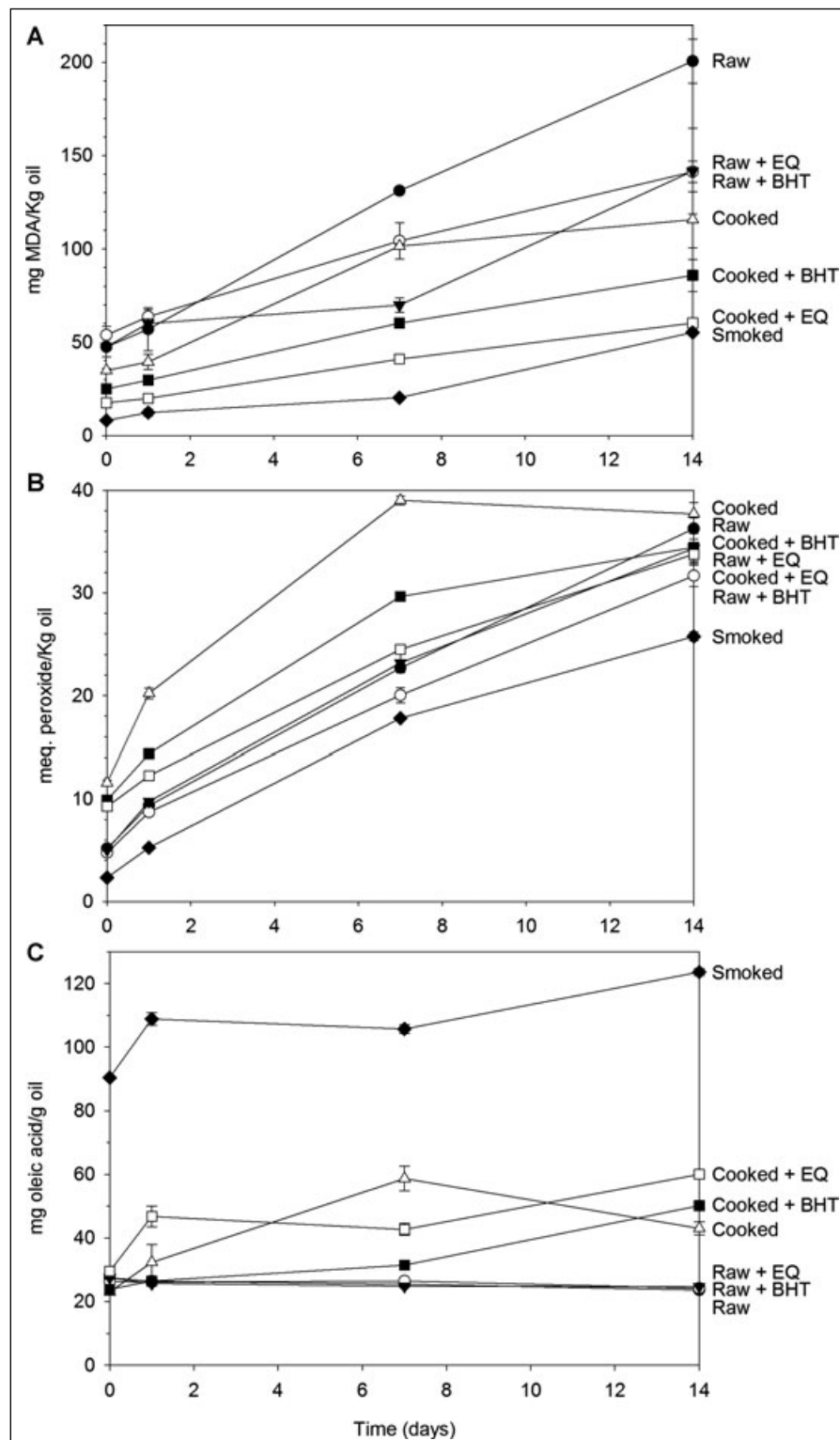

Figure 5 — Antioxidant potential of oils extracted from salmon heads that were smoke-processed (5 h) at different temperatures.

Figure 7 — Antioxidant potential of extracted salmon oils during 30 days of storage: raw oil 4 °C (●), raw oil 35 °C (○), smoked oil 4 °C (▼), and smoked oil 35 °C (△).

processing salmon heads prior to extracting the oil dramatically increased antioxidant capacity to values ranging from 8 to 12 nmol Trolox Eq/mg of oil. Antioxidant values for these oils decreased when the salmon heads were heat-processed above 75 °C. This loss may be due to direct thermal decomposition of the antioxidant compounds. Alternately, increased usage of available antioxidants may be occurring as they react with breakdown products to offset the accelerated rate of oil oxidation caused by the heat.

Salmon heads that were smoked and then stored for up to 6 d at either 4 °C or 35 °C before oil extraction had more antioxidant potential than their raw counterparts (Figure 6). A lower storage temperature (4 °C) appeared to enhance the antioxidant capacity for smoked samples, as compared to warmer storage conditions (35 °C). When oils were extracted from salmon heads immediately after smoke-processing and stored at 4 or 35 °C for up to 30 d, the smoked oils also displayed more antioxidant

Figure 8 – Oxidation of oils extracted from smoked salmon heads compared to oils from raw and cooked salmon heads as measured by (A) TBARS, (B) peroxide values, and (C) fatty acid values.

potential than raw oils (Figure 7). A small but significant increase in antioxidant potential was unexpectedly observed during storage of smoke-processed heads and oils. It is possible that natural or smoke-generated antioxidant compounds were initially bound, either within the smoked salmon tissue or within the extracted crude oil. In a bound state, these antioxidants would be unmeasurable. However, decomposition and other changes occurring during storage might result in release of these antioxidant compounds, thereby increasing their availability with increased storage time.
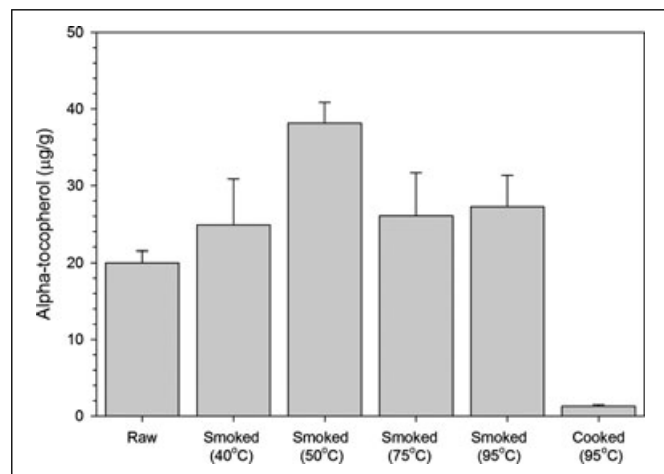
Smoked oils compared to commercial antioxidants

To gauge the practical value of antioxidants in smoke-processed salmon oils, a challenge study was initiated using commercially available antioxidants, EQ and BHT. Both EQ and BHT are fat-soluble antioxidants commonly used in the food and feed industries. Oils extracted from raw and cooked salmon heads were supplemented with commercially appropriate levels of EQ or BHT to serve as controls. TBARS values for oils extracted from raw salmon were consistently higher than the oils from either smoked or cooked salmon regardless of the presence of antioxidant

Figure 9 — Alpha-tocopherol (vitamin E) retention in oils extracted from salmon heads smoke-processed at different temperatures (40 °C to 95 °C, 5 h). Raw and cooked (95 °C, 1 h) controls are also included.

Figure 10 — Alpha-tocopherol (vitamin E) retention of raw and smoked salmon oils stored for 30 days (4 °C and 35 °C), as well as raw and smoked salmon heads stored for 6 days (4 °C and 35 °C) before oil extraction.
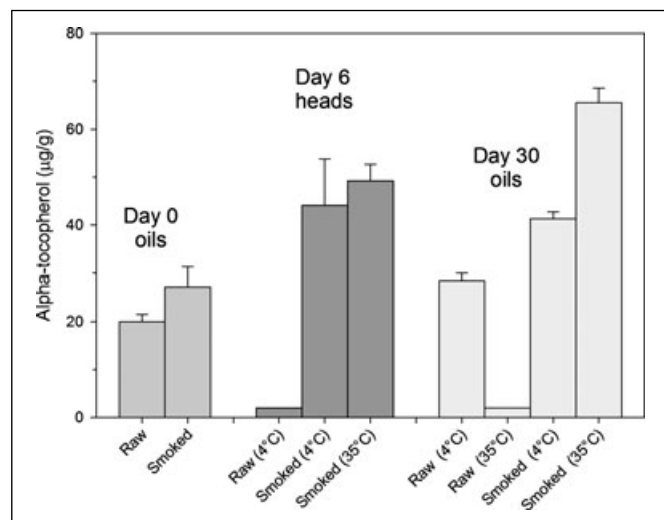
(Figure 8A). Both antioxidants tested (EQ and BHT) were able to decrease the level of oxidation (as detected by TBARS) for cooked and raw oils, with EQ in cooked oils being nearly as effective as smoked oils for inhibiting oxidation over 14 d. However, the most effective method for inhibiting lipid oxidation was achieved by smoke-processing the salmon heads before oil extraction, rather than adding EQ or BHT.

PV for oils extracted from cooked samples, regardless of the presence of antioxidant, were higher than all other samples except the raw control (Figure 8B). Antioxidant comparisons with EQ and BHT (as measured by PV) displayed a trend that was similar to the TBARS results, with oil extracted from smoked salmon heads able to inhibit oxidation to a greater extent than cooked and raw oils, even those containing EQ or BHT.

Antioxidants apparently had little effect on fatty acid quantities (Figure 8C). This is reasonable, since FA are generally the result of hydrolytic processes rather than oxidative ones. As previously noted (Figure 3C), oils extracted from smoke-processed salmon heads incurred higher levels of FA during storage than oils extracted from either cooked or raw salmon.

Vitamin retention of smoke-processed oils

In nature, lipid-containing tissues are typically associated with natural compounds, such as fat-soluble vitamins and carotenoids, which inhibit lipid oxidation. When less oxidation occurs, naturally occurring antioxidants are preserved. Oils extracted from raw salmon heads contain vitamin E (α-tocopherol), which was destroyed by cooking, but apparently enhanced through smoke-processing (Figure 9). The open-vat cooking process involved grinding the salmon heads prior to heating, which increased the surface area of the tissue, thereby exposing more of the lipids to oxidation. The smoking process likely protected the salmon head oils by imparting antioxidant compounds known to be present in smoke (Pearson and Gillett 1996), thereby allowing the oils to withstand vitamin E loss during the grinding procedure associated with crude oil extraction. Protection of vitamin E levels was also noted in smoked salmon heads stored for 6 d prior to extraction (Figure 10). Loss of vitamins in fish was briefly reviewed by Aminullah-Bhuiyan and others (1993), noting that smoke-processing did not result in appreciable losses of oil-soluble vitamins in fillets of mackerel.

Protective effects of smoke-processing

Heating disrupts salmon tissues, thereby facilitating contact between the PUFA-rich oils and prooxidants in the cells. Lipid peroxidation is controlled by endogenous factors (such as lipid quantity and composition, pro-oxidants, and antioxidants present), as well as exogenous factors (such as oxygen, heat-processing, and storage). Exposure to high temperatures may also decrease salmon protein's capacity to bind pro-oxidant transition metals, leading to oxidation (Let and others 2007). Oxidative stabilities of PUFAs were also found to be enhanced in aqueous environments (Kobayashi and others 2003), compared to nonpolar solutions. The protected environment within the smoked salmon heads contained higher moisture levels than the exposed outer layers and may have also contributed to the increased stability of smoke-processed oils.

Conclusions

This study demonstrates that smoking salmon heads prior to oil extraction protects valuable PUFAs from oxidation and imparts antioxidant compounds to the oils. However, temperatures above 75 °C decreased the antioxidant capacity of smoke-processed oils. In addition to providing a unique smoke-flavored additive for inclusion in foods, smoke-processing can extend the shelf life of fish,

thereby offering processors the additional time needed to extract valuable marine oils.

Acknowledgments

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